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RELATIONSHIP BETWEEN IMMUNE CELL PHENOTYPES AND PIG GROWTH IN A COMMERCIAL FARM

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The objective of this study was to evaluate the relationship between the level and function of circulating immune cells with average daily gain, live and carcass measurements, feed intake, and feed conversion. Production performance was monitored throughout the pig's lifetime. Pigs were moved in weekly batches through the nursery and growing/finishing rooms at specific target weights. Animals were individually weighed at birth and at weaning, and then every two weeks while they were "on test" until they were "off test" and sent to the slaughterhouse. At six to seven weeks of age, the pigs were bled in the nursery. The percentage of immune cell subsets and lymphocyte proliferation was estimated using swine monoclonal antibodies and flow cytometric analysis. The predictive effect of the immune cell subset markers and lymphocyte proliferation on production traits was statistically analyzed. The results indicated that the proportion of several peripheral cell subsets, including CD16+, CD2+/CD16+, and CD8+ lymphocytes, appear to predict growth during the entire productive life of the pig. Larger percentages of lymphocytes expressing CD16+ CD2+/CD16+, and CD8+ receptors in blood resulted in a reduction in average daily gain. In addition, high percentages of SLA-DQ+ cells were associated with better carcass weight and feed conversion. The CD16+, CD2+/CD16+, CD8+, and SLA-DQ± cell subsets appear to be important biomarkers involved with the inherent ability of the pig to efficiently grow and produce better carcass weight in representative commercial environments.

Keywords: Immune biomarkers; Immune response; Pig robustness

INTRODUCTION

Genes, gene products, and cell types involved in the response to environmental challenges may play an important role in pig growth and other production traits and could be targets for genetic selection. Cells involved in the function of the immune system may be of particular interest (1). Immune cell numbers and functions show considerable genetic variation in outbred pig populations (2–8), and it is possible that this genetic variation influences animal production traits.

Genetic selection of animals based on immunocompetence has shown variable results. Negative relationships between growth rate and antibody production (9–10)

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and high body weight and immune performance (11–12) have been reported in poultry. In pigs, detrimental relationships between lean growth and immunocompetence have been documented (13–15). In trials of pigs with lean and fat genotypes, similar mortalities were observed in a biosecure, low pathogen stimulation environment (segregated early weaning, and three-site production), but differences were found in mortality in a less biosecure, high pathogen stimulation environment (conventional weaning and continuous flow finishing) (16).

In an example of genetic selection and immunocompetence, quantification of somatic cell counts (SCC), a group of cells made up mainly of leukocytes, is commonly used as a measure of milk quality in dairy cows. Studies have shown high genetic correlations between SCC and clinical mastitis, and bacterial infection (17–19). Clinical mastitis and bacterial infection both have low heritability and are difficult to score, whereas heritability for SCC is moderate and easy to score. SCC counts are used as additional criteria for animal selection in dairy cows. This is an example where an association between immune cell numbers and a production trait has been exploited commercially.

In yet another example, the association between productivity traits and lymphocyte subsets and numbers of white cells was studied in 24-week-old pigs (20). Results of that study using a small number of pigs suggested that higher proportions of certain lymphocyte subsets, including CD8 and natural killer (NK) cells, had a negative relationship with live weight.

Knap and Bishop (21) reviewed reports on genetic selection and immunocompetence and suggested that many studies derive their contrasts from comparisons of different breeds, or at best from selection lines that were developed from the same base population. They cautioned that it is important to quantify genetic variation within breeds or genetic lines, rather than contrasting breeds.

The goal of this study was to understand if the frequency and function of immune cells influence pig production parameters. Associations between the level and function of circulating lymphocytes and production parameters were investigated within pig lines, throughout the productive life of the pig and under commercial environments.

MATERIALS AND METHODS

Animals

The production performance of 140 pigs was monitored from September 2000 to April 2001 (Study 1). In a follow-up study, one year later, 225 pigs from the same farm were also monitored. The pigs were a product of three different commercial lines derived from Large White, Landrace, Pietrain, Duroc, and Hampshire breeds. The overall male-female ratio in the population was 1–1.3. The number of sires and dams used to produce the offspring for these studies is shown in Table 1.

Experimental Design

The plan for the experiments is presented in Figure 1. Pigs were born on farm A, site I, which comprises the breeding, gestation, and farrowing units. All pigs were processed 24 hours after birth: each pig was individually weighed (Mosdal scale

Table 1 Number of sires and dams that were used to produce the offspring in the original (Study 1) and follow-up studies

SireLine\DamLine	Commercial Line A	Commercial Line B
Commercial Line X (10 sires in study 1)	19	61
Sire Line Y (8 sires in initial study and 15 in follow-up study)	100	42

model IQ-plus 390-DC), identified with button ear tags, and given a 1 cc mixture of iron dextran (Durvet Inc.), penicillin (PFI.PenG, Pfizer), and 1 cc of gentamicin (Garacin, Schering Plough Laboratories) for scour prevention. At weaning (±19 days of age), each pig was individually weighed, color tagged, and transported to a nursery on site II, where they were housed for at least seven weeks. Pigs were moved in weekly batches through the farrowing, nursery, and growing/finishing rooms, moving to the next production stage when they met specific target weights. There were nine nurseries, and pigs were penned by sex and weight for seven weeks, and housed equally distributed with other nonexperimental pigs of the same age and commercial genotype. A total of 15 pigs per pen was targeted. A four-stage nursery nutritional program was implemented, as well as a rigorous feed budget program. In the pre-nursery and nursery diets, 200 g/ton of neomycin sulfate and oxytetracyclin (Neo/Terra, Pfizer Animal Health) were used. Pigs were vaccinated at 10 and 24 days of age with a *Haemophilus parasuis* bacterin (Suvaxyn RespiFend HPS, Fort Dodge Laboratories). At nine weeks of age, pigs were weighed, and animals that reached the target weight of 32 kg were put "on test" by moving them out of the nurseries into the growing/finishing rooms. Two to four nursery rooms were used to fill one growing/finishing room. Pigs were housed with other nonselected pigs

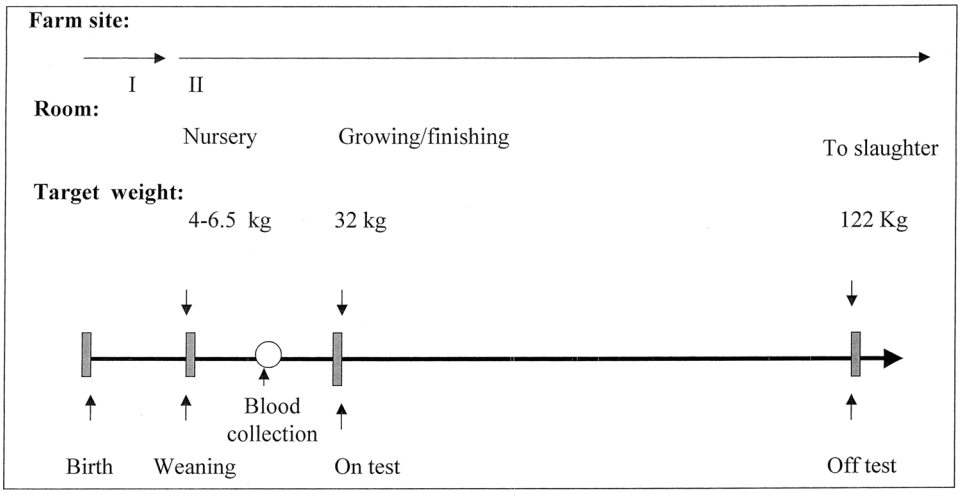

Figure 1 Experimental timeline describing key features of the study including farm site, production stage, target weight, and collection of blood for determination of % of immune cells and functional assays.

of the same age and genotype. Randomly selected pigs were equally represented in each pen. Barrows and gilts were distributed over eight pens within a room in the finishing building. Room temperature variation was minimal and ranged from 21.6°C high and 19.4°C low. Pigs were fed with a four-phase corn-soy–based diet program. Target weight for weaned pigs was 4 kg, for the "on test" period it was 32 kg, and to be "off test" it was 122 kg.

Growth and Production Traits

The testing period included the time when pigs were out of the nurseries and were put on test until the time they were taken off test. Pigs were individually weighed on test (True-Test model 700 scale) every two weeks until the pigs reached the target off-test weight of 122 kg. Average daily gain (ADG) was calculated for different time periods: (a) on test, (b) from birth to weaning, (c) birth to off test, also referred to as lifetime daily gain (ldg), (d) weaning to off test, and (e) weaning to on test. Individual feed intake was measured using a FIRE system (Osborne Industries, Osborne, KS) using transponders in each pig, and feed conversion was calculated. In addition, live body measurements were taken, including back fat at the first rib (frstrib), last rib (bf), last vertebrae (Lvert), and loin depth (LD) with a real time ultrasound (Aloka model SSD-500 V). At the end of the off-test period, pigs were transported and slaughtered at Swift slaughter facilities (Louisville, KY). Carcass traits collected included hot carcass weight, back fat, and loin depth, measured by a fat-o-meter (SFK Technology Inc., Peosta IA), and percent lean.

Farm Health Status

A veterinarian assessed clinical disease and mortality at monthly visits to the nursery and growing/finishing units. The farm was considered to have good health status with limited clinical signs of disease. Historically, pigs in farm A had a low to medium level of *Salmonella* exposure determined by serology (Danish Mixed ELISA at Iowa State University). *Mycoplasma hyopneumoniae* seroconversion (Tween-ELISA test at the University of Minnesota Diagnostic Laboratory) was demonstrated during the study.

Blood Collection

Blood was obtained at six to seven weeks of age by puncturing the anterior vena cava and collected in two 10 ml tubes containing anticoagulant (EDTA). Blood was kept on ice and sent by courier to MD for laboratory processing the next day.

Cell Preparation

Peripheral blood mononuclear cells (PBMC) from all pigs were isolated by gradient separation using lymphocyte separation media (LSM, Cappel, ICN Biomedicals, Ohio) as described elsewhere (22). After PBMC isolation, cells were counted using Trypan blue dye exclusion and their live cell concentration adjusted to 5×10^7 cells/ml.

Immunostaining and Flow Cytometry

After isolation of PBMC from whole blood, lymphoid cells were tested under two conditions. First, aliquots of PBMC were tested directly and the percentages of immune cell subsets determined by immunostaining the PBMC with monoclonal antibodies (mAbs) against swine cell subset, or CD, antigens (Table 2). Second, aliquots of PBMC were cultured with the mitogen Concanavalin A (Con A, Sigma, St. Louis, MO) after staining with PKH67 (Sigma, St. Louis, MO) and proliferation response of cell subsets determined using FCM and combined mAbs (22). All monoclonal antibodies used in this study were specific for swine and their specificity confirmed in international workshops (23–24). These mAbs were chosen because they target surface markers that are expressed on PBMC monocytes and lymphocytes. An anti-swine pan leukocyte marker (CD45) and multiple IgG isotype control mAbs were used as positive and negative controls, respectively. The percentage of cells immunostained with the specific mAbs was determined after labeling cells with secondary $F(ab)'_2$-anti-Ig isotype antibodies coupled with FITC or PE (Southern Biotechnology Associates, Birmingham, AL).

Table 2 Swine monoclonal antibodies and target cell in blood mononuclear cells

Cell subset	Monoclonal antibody	Target cell
CD4	74-12-4	T lymphocyte subset that reacts with MHC type II antigen and foreign antigen on antigen presenting cell (APC)
CD8	76-2-11	T lymphocyte subset that reacts with MHC type I antigen and foreign antigen on APC
CD4+ CD8+		Double positive T lymphocytes expressing both CD4 and CD8; normally dull CD8
CD172	74-22-15	Monocytes, macrophages, granulocytes; for PBMC reacts primarily with monocytes; previously referred to as SWC3 marker
SLA-DQ-T	TH-16	Subset of swine lymphocyte antigen (SLA) class II genes. Detects and SLA-DQ positive lymphoid cells.
SLA-DQ-B		SLA-II B gated to identify only SLA-DQ bright lymphocytes and/or macrophages
SLA-DQ-D		SLA-II D gated to identify SLA-DQ dull lymphocytes and/or macrophages
CD2	MSA3	Sheep red blood cell receptor; predominantly expressed on T and NK cells; dull on B cells
CD16	G7	Low affinity receptor FCR I II; expressed by multiple cell subsets; NK, B, macrophages
CD2+/CD16+		Double positive lymphocytes expressing both CD2 and CD16; in pigs, the natural killer (NK) cells.
CD21	IAH CC51	B lymphocytes
CD45	K252 1E4	Panleukocyte marker used as positive control
IgG1 control	MCA 1321	Anti-neurofilament 200 kd; does not bind pig cells; used as IgG1 negative control
IgG2a control	MCA 1558	Anti PcNA; does not bind pig cells; used as IgG2a negative control
IgG2b control	MCA 490	Anti-RSV fusion protein; does not bind pig cells; used as IgG2b negative control

Immunostained PBMC were analyzed using flow cytometry (FCM) as described elsewhere (22). The percentage of stained cells was calculated based on the fluorescence intensity using the IgG isotype background as a control. For certain markers it was important to distinguish between dull (D) and bright (B) immunofluorescence; thus, for swine leukocyte class II (SLA II), or SLA-DQ, the total (T), D and B populations were reported. The FCM analysis for each sample used light scatter, based cell bitmaps, analyzing 16,000 to 20,000 events.

Lymphocyte Proliferation

PBMC were washed once in RPMI 1640 medium, followed by filtering through a nylon mesh in a 17×100 ml polypropylene conical tube. The technique was adapted from previously published techniques but using PKH67 staining (25,26). The cell pellet was taken up in Diluent C (Sigma, St. Louis, MO) to achieve a final suspension of 5×10^6 cells/ml. This cell suspension was then added to an equal volume of PKH67 dye stock (1.5 µl dye/1×10^7 cells) and incubated 3 min at room temperature. Cell concentrations were previously established to stain cells homogenously and bright. A volume of heat-inactivated fetal bovine serum (FBS) equal to the total volume of cells and dye was added to the suspension. The cells were then centrifuged three times, washed the first time with RPMI medium with serum and the final time in blastogenic media (RPMI 1640 supplemented with 5% FBS, 1 mM sodium pyruvate, 2 mM L-glutamine, nonessential amino acids, 5.5×10^{-5} M 2-mercaptoethanol, 25 mM Hepes buffer), and counted with a hemocytometer. Homogeneity of the staining procedure was checked by FCM on day 0 for an aliquot of these labeled cells. A total of 8×10^6 PKH67-labeled cells were plated into individual wells of a 24-well tissue culture plate. A final dose of 5 µg/ml ConA was added to the wells at the beginning of the culture period, while control wells had medium without mitogen. The final volume was 2 ml/well. The plates were incubated at 37°C in 5% CO_2 for three days. The top 1 ml of medium was exchanged with 1 ml of fresh blastogenic medium in wells after 48 h of culture. On day 3, the cells were collected with two washes in RPMI medium and counted. A concentration of 1×10^7 cells/ml was used for differential immunostaining as described by Solano-Aguilar (22). Afterward, culture cells were stained with control mAbs or CD4 or CD8 mAbs.

Date collected were used to calculate lymphocyte proliferation after Con A exposure as a measurement of lymphocyte function. Proliferation of CD4 positive (CD4+), CD8 positive (CD8+), and PKH67 incorporation (total cell proliferation) were compared between cells cultured with Con A or with media to determine if any cell subset was preferentially increased after stimulation. Flow cytometry data were analyzed with the Proliferation Wizard module in Modifit Lt software (Verity Software House, Topsham, ME). Cells were gated according to the forward and side scattered signals of the lymphocyte population to exclude debris. The intensity of the nonproliferating (parental) cells was determined by analysis of the sample that had been cultured without mitogen. Each generation of cells should have approximately half the PKH67 dye of the parental cells. Working down from the intensity of the parental generation, the Modifit software deconvolutes the fluorescence intensity histogram with Gaussian distributions centered on the peak at different channel intervals (26). Using the data returned by the software for the percentage of cells in

each daughter generation at the time of the analysis, the individual frequency of each cell subset (CD4+, CD8+, PKH67+) in the original population that had proliferated (precursor frequency or PF) was calculated. The proliferation index (PI) was calculated as the sum of the cells in all generations divided by the number of original parent cells theoretically present in the nonstimulated population. The PI is a measure of the increase in cell number in the culture over the course of the experiment (three days). Precursor frequencies and proliferation indexes were used as measurements of lymphocyte function in this study.

Statistical Analysis

Effects of immune phenotypes and proliferation assays on production parameters were estimated. A mixed model (SAS Proc. MIXED, SAS Institute Inc., Cary, NC, USA) was used to determine which parameters should be used in addition to the predicting effect when estimating the effects on growth traits. The full model used included commercial genetic line, sire (nested within commercial line), nursery room, finishing room, sex, and parity of sow, sire was a random effect and all the others were fixed. The predictive effect of immune cell subset markers and proliferation assays on production traits were estimated with the following models:

Growth: Imm + sire (commercial line)+ nursery room+ sex+ parity
Live carcass weight: Imm + sire (commercial line) + nursery room + sex + parity+ liveweight
Feed parameters: Imm + sire (commercial line) + nursery room + parity,

where Imm is the immune parameter investigated and liveweight is the weight of the animal when the immune parameter was measured.

In the follow-up study one year later, 225 pigs from the same genetic background and the same farm were tested, under the conditions described above, for associations between lifetime daily gain and the frequency of selected lymphocyte subsets including CD2+/CD16+, CD4+, and CD8+ cells.

RESULTS

The average weaning weight was 6.4 kg ± 0.2 (19.4 days ± 0.2). At weaning, 76% of the piglets fell within the 4–6.5 kg weight range; of the rest, 1% of the pigs fell below a weight of <4 and 23% were >6.6 kg. The average initial on test weight was 33.3 kg ± 0.5 (70.8 days ± 0.6). Average off-test weight was 123.1 kg ± 0.7 (166.8 days ± 0.9).

Veterinary reports indicated that some pigs showed clinical disease in the nurseries. Clinical signs included occasional weak pigs that did not develop well, and coughing. Overall nursery and growing/finishing mortalities were 1–2% and 2%, respectively. These percentages are within low to normal ranges of mortality rates in commercial operations. The mortality parameters and growth rates for this farm indicate that the production and health status of the farm were adequate and stable (i.e., represented stable commercial environments).

The immunostaining and FCM results obtained with the CD45 panleukocyte mAb showed that an average of 92.04% of the cells isolated were recognized by the CD45 mAb as leukocytes, indicating an appropriate lymphoid gate. Background staining was controlled by the use of IgG isotype controls, which were present at a desirable $< 5\%$. All mAbs used showed higher values than the background controls, indicating that the detection system was effective. The data analyzed by FCM with the Modifit program indicated that 99.37% of cells were stained with PKH67, demonstrating the high efficiency of the procedure.

Typical flow cytometry results for pig 1867 are shown in Figure 2. The average peripheral blood cell subsets, indicated by their CD markers, are noted in Table 3.

Significant associations were found between immune markers and production at the stage that covered the period when the blood sample was collected (nursery). Additionally, associations were found with other production stages, suggesting that these markers predict productive performance through the entire life of a pig.

Three phenotypes were significantly associated with ADG during the lifetime of the pig, including CD16+ cells ($p < 0.05$), CD2+/CD16+ cells ($p < 0.01$), and

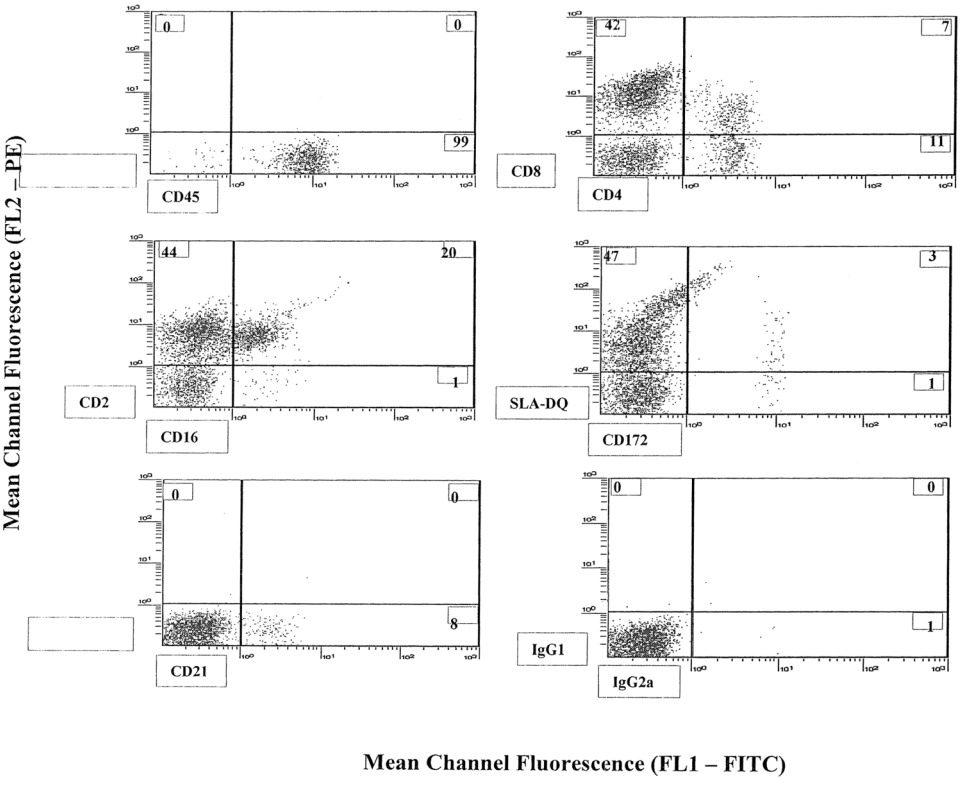

Figure 2 Cell subset immunostaining for pig 1867. Cells were stained with the specific monoclonal antibodies (MAb) including CD45, CD4+/CD8+, CD16, etc., followed by PE- or FITC-labeled anti-isotype Abs, and analyzed by flow cytometry. Numbers in each quadrant indicate the % of cells with that set of PE- and FITC-fluorescent intensities.

Table 3 Average percentage blood mononuclear cell subsets

Cell subset	Average % in blood*
CD4+ T	14.6 ± 5.5
CD8+ T	40.2 ± 14.8
CD4 + CD8+ T	8.4 ± 3.3
CD172+ Monocytes	3.9 ± 3.3
SLA-DQ-T (Total class II+)	48.8 ± 11.2
SLA-DQ-B (Bright class II+)	26.8 ± 9.7
SLA-DQ-D (Dull class II+)	22.0 ± 8.8
CD2+	60.0 ± 17.8
CD16 (FcR+ cells)	20.0 ± 9.4
CD2+/CD16+ NK	18.0 ± 9.2
CD21+ B	12.0 ± 5.2
CD45	K252 1E4
IgG1 control	MCA 1321
IgG2a control	MCA 1558
IgG2b control	MCA 490

*% and standard deviation blood cell subset prepared as described in "Materials and Methods."

CD8+ cells ($p < 0.05$) (Table 4). A functional assay, the CD4 PF, was also associated with ADG during the lifetime of the pig ($p < 0.1$) (Table 4). These four phenotypes were negatively correlated with growth; higher percentages of these cell subsets were associated with lower ADG.

Results of the statistical analysis indicated that each 1% increase of CD2+/CD16+ cells is predicted to create a reduction of 18 grams of lifetime daily gain. To assess the impact of frequency of CD2+/CD16+ cells on lifetime ADG, weight gain and the average number of days to market was compared between the 30 pigs showing the highest percentages and 30 pigs showing the lowest percentages of CD2+/CD16+ lymphocytes. The bottom 30 responders had an average of 5.4% of CD2+/CD16+ cells, and the top 30 had an average of 33.1%. This means that the predicted difference in lifetime gain is $(33.1 - 5.4) \times 0.018\,\text{kg/day} = .0498\,\text{kg/day}$. For the average 167 days of age for a pig in this study, the predicted difference high and low % of CD2+/CD16+ lymphocytes on end weight was 8.32 kg.

Some lymphocyte subsets and proliferation assays were significantly associated with ADG at specific production stages. CD4+ cells were associated with ADG from birth to weaning ($p < 0.05$), SLA-DQ-B+ cells were associated with ADG from weaning to on test ($p < 0.1$), and CD172+ cells were associated with ADG on test ($p < 0.1$) and from weaning to off test ($p < 0.1$). All the associations had positive correlations; the higher the percentage of cells, the greater the ADG (Table 4).

Associations were found between CD4+/CD8+ cells and back fat (Lvert) at on test ($p < 0.05$), CD8+ PF and first rib at off test ($p < 0.1$), and SLA-DQ-D+ and back fat at on test ($p < 0.1$) (results not shown). However, those associations were not significant when the same traits were evaluated on the carcass instead of the live pig. Furthermore, the proportion of SLA-DQ-B+ and SLA-DQ-T+ cells were associated with hot carcass weight ($p < 0.05$). The correlation was positive; higher proportions of SLA-DQ-B+ and -T+ were associated with greater hot carcass weight (results not shown).

Table 4 Effect of the proportion of immune cell subsets and proliferation responses on average daily gain (ADG). Significant ($p < 0.10$) regression coefficients are shown in bold. Only cell subsets and lymphocyte proliferation responses (PF) with at least one significant ADG trait are shown. Results from 120–140 observations from Study 1 are included

Cell subset	CD16		CD2/CD16		CD4		CD8		CD4 PF delta		SLA-DQB		CD172/SWC3	
Dependent trait	Reg estimate (s.e.)[@]	p*	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p
ADG on test	−.004	>0.1	**−.005**	<0.05	−.002	0.64	−.002	>0.1	−.099	>0.1	−.003	>0.1	**.0137**	<0.1
	(.0023)		**(.0023)**		(.0035)		(.0015)		(.0932)		(.0025)		**(.0073)**	
ADG birth to weaning	**−.002**	<0.05	**−.002**	<0.05	.003	**0.04**	−.001	>0.1	−.013	>0.1	−.001	>0.1	−.004	>0.1
	(.0009)		**(.0009)**		(.0014)		(.0006)		(.0494)		(.0010)		(.0029)	
ADG lifetime	**−.003**	<0.05	**−.004**	<0.005	−.002	0.39	**−.002**	<0.05	**−.109**	<0.1	.0005	>0.1	.0074	>0.1
	(.0014)		**(.0014)**		(.0022)		**(.0009)**		**(.0624)**		(.0016)		(.0047)	
ADG weaning to off test	**−.003**	<0.05	**−.004**	<0.01	−.002	0.3	**−.002**	<0.05	**−.105**	<0.1	.0006	>0.1	**.0079**	<0.1
	(.0014)		**(.0014)**		(.0021)		**(.0009)**		**(.0605)**		(.0015)		**(.0046)**	
ADG weaning to on test	−.006	0	**−.006**	<0.005	−.003	0.39	**−.004**	<0.005	**−.141**	<0.1	**.0037**	**<0.1**	−.002	>0.1
	(.0019)		**(.0019)**		(.0030)		**(.0012)**		**(.0818)**		**(.0021)**		(.0063)	

@ = Regression estimate (standard error).
* = p value.

Table 5 Effect of the proportion of immune cell subsets and proliferative responses on feed intake and feed conversion. Significant ($p < 0.10$) regression coefficients are shown in bold. Only cell subsets and proliferative responses with at least one significant value are shown. Results from 120–140 observations from Study 1 are included

Cell subset	SLADQ-D		SLADQ-T		CD172/SWC3		CD4	
Dependent trait	Reg estimate (s.e.)[@]	p^*	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p	Reg estimate (s.e.)	p
Feed intake	**.0109 (.0053)**	<0.05	**.0057 (.0029)**	<0.1	**.0274 (.0135)**	<0.05	**−.011 (.0061)**	<0.1
Feed conversion	**.0111 (.0058)**	<0.1	**.0078 (.0031)**	<0.05	.0245 (.0148)	>0.1	−.011 (.0066)	>0.1

@ = Regression estimate (standard error).

* = p value.

Feed intake was significantly associated with the proportion of SLA-DQ-D+ ($p < 0.05$), SLA-DQ-T+ ($p < 0.1$), CD172+ ($p < 0.05$), and CD4+ ($p < 0.1$) cells. SLA-DQ-D+ ($p < 0.1$) and SLA-DQ-T+ cells ($p < 0.05$) were also associated with feed conversion (Table 5).

In the follow-up study of 225 pigs, lifetime daily gain was also associated with the frequency of CD2+/CD16+ lymphocytes ($p < 0.1$), CD4+ ($p < 0.1$), and CD8+ lymphocytes ($p < 0.005$) (results not shown). These results are in agreement with the initial results from the 140 pigs studied the previous year at the same farm.

DISCUSSION

The frequency of certain lymphocyte subsets in the bloodstream appeared to influence growth, feed efficiency, and carcass traits in the commercial pigs of this study. To understand the nature of the associations, it is helpful to review the function of these cell subsets. Significant associations were found between CD2+/CD16+ lymphocytes and lifetime daily gain. Natural killer cells are CD2+/CD16+ lymphocytes that lyze target cells without prior sensitization or major histocompatibility complex (MHC) restriction, and therefore this response is not antigen-specific. Natural killer cells respond to a wide range of microbes, they have antiviral and antineoplastic activities, and they are involved in regulation of hematopoiesis. Recent data indicate that NK cells are critical regulators of innate and adaptive immunity through their Toll-like receptors (TLRs) and interactions with dendritic cells (DCs) (27–30). The NK cell function is regulated by interleukin-2 (IL-2), IL-4, interferon-γ (IFN-γ), and by a fine balance between signals transmitted by activating receptors, which recognize ligands on different cells (30–32). In turn, NK cells influence other cells, particularly DCs, thereby influencing the direction and intensity of the resultant adaptive immune response (27–32).

Under resting conditions, peripheral NK cells express few cytokines; but they can be induced through their TLRs to express other immune molecules, e.g., granulocyte-macrophage colony-stimulating factor (GM-CSF), M-CSF, IL-3, IFN-γ, tumor necrosis factor-α (TNF-α), and Transforming Growth Factor-β (TNF-β) (29). Soluble mediators such as TNF-α and IFN-γ, secreted by activated NL cells, play important roles in the induction of apoptosis and additional regulation of immune responses, respectively. Therefore, the associations between NL and other lymphocyte subsets and production traits may be influenced by the presence of other cells or molecules that either regulate or are regulated by them or by the pathogens they encounter (30–32). Results from this study suggested that smaller proportions of circulating NK are important for growth in intensive production systems and that high proportions of CD2+/CD16+ NK cells are detrimental for growth.

CD4+ and CD8+ lymphocytes were significantly associated with growth traits. CD4+ lymphocytes were associated with ADG from birth to weaning, and proliferation of CD4+ PF was associated with lifetime daily gain. CD4+ lymphocytes are part of the antigen-specific immune response. CD4+ lymphocytes are mostly helper cells that recognize peptides bound to class II MHC molecules on the surface of other cells such as B cells, DCs, macrophages, and in pigs in some subsets of activated T cells. Class II associated peptides are usually derived from

extracellular microbes and soluble protein antigens (33–34). T helper (CD4+) lymphocytes can be classified as either T helper 1 (Th1) or Th2, depending on their cytokine profile and their function. Th1 cells predominantly produce IFN-γ and TNF-α and are involved in cell-mediated immunity to intracellular pathogens. Th2 cells produce IL-4, IL-5, IL-6, and IL-13 and stimulate humoral immunity and response to extracellular pathogens.

Peripheral CD8+ lymphocytes were significantly associated with lifetime daily gain. Like CD4+ cells, CD8+ lymphocytes are involved in antigen-specific responses. CD8+ cells are mostly cytotoxic T lymphocytes (CTL) that recognize peptide fragments bound to class I MHC molecules on cells that are targets of the lytic action of CTL (34). These peptides are generally derived from endogenously synthesized proteins such as viral antigens. Results of this study suggest that the frequency of CD4+ and CD8+ peripheral lymphocytes is an indicator for growth. Adequate levels of both of these antigen-specific cell subsets are important for performance, whereas exaggerated responses are detrimental for growth.

The frequency of SLA-DQ+ cells was significantly associated with carcass traits and feed conversion and feed intake. Like growth, feed conversion and carcass traits are of economic importance in the swine industry. The SLA or swine MHC molecules play an important role in antigen recognition, and they are involved in antigen-specific immune responses. Associations between SLA and disease resistance (1,35–38), reproductive performance (39,40), and production traits (41–44) have been reported. The biological process that explains those associations is not known. If the SLA proves to be useful as selection criteria, the biology of the associations with reproduction, disease resistance, and production traits should be elucidated. Because the SLA influences many important biological functions and because SLA polymorphism ensures protection against a wide variety of microorganisms, genetic selection of animals based on SLA requires deep understanding of the range of functions that may be affected.

The proportion of CD172+ cells was associated to ADG in some stages of production. The CD172 mAb targets monocytes in PBMC. Monocytes are critical for natural immunity, but they also play a central role in specific acquired immunity. Some monocyte functions include phagocytosis of foreign particles, production of mediators to kill microbes, control the spread of infections, production of cytokines and growth factors, antigen-presenting cell function, and promotion of T cell activation (34). In this study, a higher frequency of CD172+ cells was significantly associated with ADG on test and from weaning to off test and feed intake.

Several production performance traits were associated with the frequency of key immune cell subsets that act in response to viral (NK cells, CD8+ cells) and bacterial infections (CD4+, SLA-DQ+, monocytes) and that account for nonspecific (NK and monocytes) and antigen-specific (CD4+, CD8+, SLA-DQ+) immune responses. These general mechanisms of immunity are necessary to activate and control infections, and measured frequencies of these cell types seem to be associated with improved growth.

It was observed that higher frequency of lymphocyte subsets had a negative impact on production traits in pigs. It can be speculated that the energy invested to mount an immune response is taken away from the energy that should have been used for tissue growth. Further investigation is required to determine if the percentage

of immune cell subsets is inherent to the pig or is a response to microbial exposure. One possibility is that the proportions of lymphocyte subsets are a result of immune activation and varied from pig to pig due to pathogen exposure, or it could be that the proportion of cell subsets present in blood is a reflection of genetic differences in the commercial lines evaluated. From the results of this study, it is difficult to associate lymphocyte phenotypes to line or genetic effects.

Preliminary work by this group suggests that differences in the proportion of peripheral lymphocyte subsets such as CD2+/CD16+ NK cells exist even in high health status herds that have a low degree of microbial exposure (Galina, unpublished data). This may be an indication that those differences are inherent to the pig regardless of immune activation. Those observations were made in pure line and commercial crossbred half siblings sired by common boars but raised in two different environments: high health and commercial (farm A) where the extreme high and low proportions of CD+/CD16+ lymphocytes were observed (Galina, unpublished data).

High frequencies of lymphocytes have been associated with detrimental outcomes. In the human uterus, increased NK numbers appear to be associated with recurrent pregnancy loss (45). In studies where pigs were challenged with *Salmonella cholerasuis*, van Diemen et al. (46) reported that under acute challenge, lymphocyte proliferation is an indicator of resistance to salmonellosis in pigs. Higher lymphocyte proliferation responses of blood lymphocytes prior to challenge were associated with decreased resistance to *Salmonella*. In addition, higher proliferation responses were significantly associated with decreased pig growth under acute salmonellosis during the one-week postinfection period of observation. The observations from the present study that low proliferation was associated with improved growth appear to be in agreement with Van Diemen's observations, suggesting that this occurs in both acute and subclinical *Salmonella* conditions. In a similar study, a negative correlation was found between CD8 and NK cell subsets and live weight in 20 pigs selected from lean growth lines and bled at 24 weeks of age (20). With the present study, these observations are supported and expanded: a larger set of data points was studied, first in 140 and then in 225 pigs in farm A, the associations were analyzed at various production stages, and average daily gain was included in the evaluations. Notably, the associations were found under commercial conditions.

These associations were observed in an environment without major clinical signs of acute bacterial or viral infections but with a history of subclinical mycoplasmosis and salmonellosis. Management practices at farm A, including same-sex penning, feeding protocols, vaccination, and pig density, are similar to those of many commercial operations. Consequently, the farm used in this study is typical of many commercial operations. However, as many factors will differ between farms, it will be necessary to test associations between immune biomarkers and production traits in different farms in order to determine the usefulness of these immune biomarkers as general predictors of performance. For example, the frequency of immune cells may vary from farm to farm depending on the health status.

In farm A, vaccination with a *Haemophilus parasuis* bacterin was administered at 10 and 24 days of age at the pre-nursery and nursery stages. The second vaccination was given at least three weeks before blood was collected for cell characterization. Therefore, it is unlikely that vaccination had a significant impact on the immune cell populations measured at 6–7 weeks of age.

The dynamics of the immune cell populations through the life of the pig are not clearly understood. The relative percentages of CD4+, CD8+, CD4+/CD8+, and CD2+/CD16+ in the blood of pigs weaned at 3 weeks of age vary dramatically during the first weeks after birth but stabilize around 5 weeks of age and remain stable by 6 weeks of age (47,48). The dynamics of those cell populations beyond 6 weeks of age are not known, but are assumed to be at the mature level and thus less variable.

Frequency of CD16+, CD2+/CD16+, CD8+, and SLA-DQ+ lymphocyte subsets and CD4+ proliferation were associated with production traits including lifetime daily gain, and feed conversion and carcass weight. These production traits are economically important. Thus, expression of CD16+, CD2+/CD16+, CD8+, and SLA-DQ+ could be biomarkers to improve production parameters under commercial environments. Frequency of these cell subsets could be used for pig production in a similar fashion as somatic cell counts for prediction of milk quality in the dairy industry. The potential use of immune biomarkers includes the identification of fast-growing animals and reduction of size variation between groups of pigs.

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